Dihydroxylation of the Triene Subunit of Rapamycin

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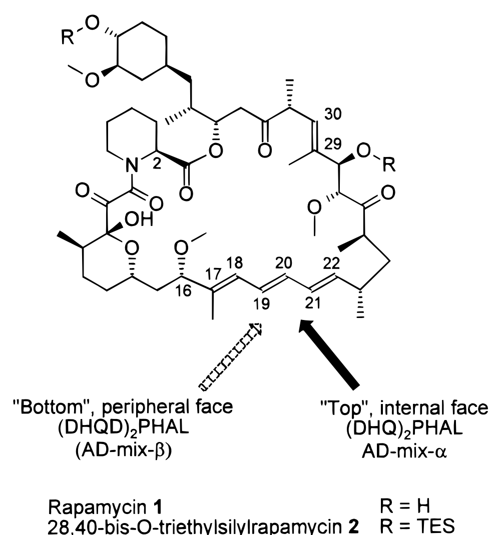
Rapamycin **1** was discovered in 1975 at Ayerst in a screen for novel antifungal agents.[1] It has attracted interest since the beginning of the nineties because of its immunosuppressive properties[2] and is presently undergoing clinical trials for the prevention of allograft rejection.[3]

The C17−C22 triene subunit represents one of the characteristic structural subunits of rapamycin. Only a few modifications of this part of the macrolide have been reported in the literature. A hetero Diels−Alder reaction with 4-phenyl-1,2,4-triazoline-3,5-dione involving C19−C22 as the diene partner has been described.[4] Two groups have published results concerning the acid-catalyzed displacement or elimination of the C16-methoxyl which in some cases occurs with concomitant rearrangement of the triene.[5] Finally, a degradation of rapamycin through exhaustive ozonolysis has been reported.[6] We were interested in exploring the selective functionalization of the triene subunit and, with this goal in mind, turned our attention to dihydroxylation. Further impetus for exploring this reaction was provided by a recent report describing a 19,20-dihydroxyrapamycin as one of the metabolites obtained by incubation of rapamycin with dexamethasone-induced rat liver microsomes,[7] though the absolute configurations of the newly introduced hydroxyls were not reported. The problems we expected at the outset of our investigation concerned the regio- and chemoselectivity. It was for instance not clear whether dihydroxylation of one of the double bonds of the triene would be preferred over oxidation of the C29,C30 olefin. Also, we would have to avoid polyhydroxylation of the substrate.

The stereoselectivity of the reaction, on the other hand, was considered to be predictable. The three-dimensional structure of rapamycin has been determined by single-crystal X-ray analyses of the macrolide alone[8] and of the FKBP12/rapamycin complex.[9] Its conformation in solution in DMSO has also been reported.[10] The conformations of the macrocycle are virtually identical in the three cases. These structures clearly show that the “bottom” face of the C19−C22 triene is exposed and accessible to peripheral attack, while the approach of any reagent on the internal, “top” face is highly unlikely (Figure 1). On the basis of this structural information we anticipated that facial selectivity would not be an issue for the dihydroxylation if the reaction were to occur in the triene subunit.



We initially resorted to modifications of the Upjohn osmylation procedure[11] using catalytic osmium tetraoxide and various amounts of NMO. These attempts met with little success. When 1 equiv of NMO was used, a mixture of rapamycin, together with dihydroxylated and tetrahydroxylated derivatives was obtained. Each of the latter consisted of a mixture of isomeric compounds which were not further characterized. The use of 2 equiv of NMO afforded an inseparable mixture consisting predominantly of tetrahydroxyrapamycins according to MS. The problems encountered with this procedure led us to contemplate the use of the asymmetric dihydroxylation (AD) conditions. Indeed, reports by several groups have documented that the AD process allows for the selective dihydroxylation of polyenes.[12−14] Most relevant for our purpose were publications from the Sharpless group demonstrating that “mono”-dihydroxylation can also be achieved in the case of conjugated polyenes.[12a,c] This

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(1) (a) Vézina, C.; Kudelski, A.; Sehgal, S. N. *J. Antibiot.* **1975**, *28*, 721−726. (b) Sehgal, S. N.; Baker, H.; Vézina, C. *J. Antibiot.* **1975**, *28*, 727−732.

(2) Dumont, F. J.; Su, Q. *Life Sci.* **1996**, *58*, 375−395.

(3) (a) Shoker, A. S. *Drugs Today* **1997**, *33*, 221−236. (b) Kahan, B. D. *Transplant. Proc.* **1997**, *29*, 48−50.

(4) Ocain, T. D.; Longhi, D.; Stefan, R. J.; Caccese, R. G.; Sehgal, S. N. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 1340−1346.

(5) (a) Grinfeld, A. A.; Caufield, C. E.; Schiksnis, R. A.; Mattes, J. F.; Chan, K. W. *Tetrahedron Lett.* **1994**, *35*, 6835−6838. (b) Luengo, J. I.; Konialian-Beck, A.; Rozamus, L. W.; Holt, D. A. *J. Org. Chem.* **1994**, *59*, 6512−6513.

(6) Goulet, M. T.; Boger, J. *Tetrahedron Lett.* **1990**, *31*, 4845−4848.

(7) Nickmilder, M. J. M.; Latinne, D.; Verbeeck, R. K.; Janssens, W.; Svoboda, D.; Lhoëst, G. J. J. *Xenobiotica* **1997**, *27*, 869−883.

(8) Swindells, D. C. N.; White, P. S.; Findlay, J. A. *Can. J. Chem.* **1978**, *56*, 2491−2492.

(9) (a) Van Duyne, G. D.; Standaert, R. F.; Schreiber, S. L.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 7433−7434. (b) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *J. Mol. Biol.* **1993**, *229*, 105−124.

(10) Kessler, H.; Haesner, R.; Schüler, W. *Helv. Chim. Acta* **1993**, *76*, 117−130.

(11) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973−1976.

(12) (a) Xu, D.; Crispino, G. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1992**, *114*, 7570−7571. (b) Xu, D.; Park, C. Y.; Sharpless, K. B. *Tetrahedron Lett.* **1994**, *35*, 2495−2498. (c) Becker, H.; Soler, M. A.; Sharpless, K. B. *Tetrahedron* **1995**, *51*, 1345−1376.

(13) Corey, E. J.; Noe, M. C.; Shieh, W. C. *Tetrahedron Lett.* **1993**, *34*, 5995−5998.

(14) (a) Vidari, G.; Dapiaggi, A.; Zanoni, G.; Garlaschelli, L. *Tetrahedron Lett.* **1993**, *34*, 6485−6488. (b) Vidari, G.; Giori, A.; Dapiaggi, A.; Lanfranchi, G. *Tetrahedron Lett.* **1993**, *34*, 6925−6928.

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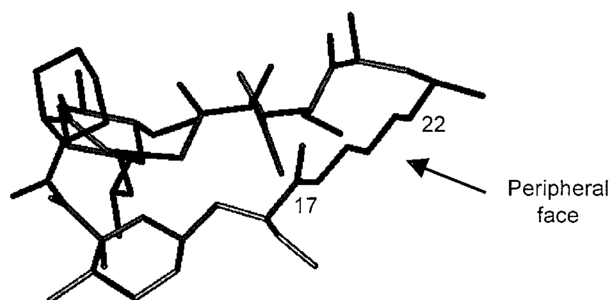


Figure 1. X-ray crystal structure of rapamycin showing that only one face of the triene subunit is accessible.

selectivity is ascribed to the preclusion of the so-called "second cycle".[12a,15] The Sharpless mnemonic device[16] indicated that the dihydroquinidine-derived ligand $(DHQD)_2$PHAL, corresponding to AD-mix-β, would promote dihydroxylation of any double bond within the triene from its accessible, i.e., peripheral ("bottom") face (vide supra). Exposure of 28,40-*O*-bis(triethylsilyl)rapamycin **2**[17] to modified AD conditions[18] using $(DHQD)_2$-PHAL resulted after 12 h at 0 °C in complete consumption of the starting material and the clean formation of the diols **3** and **4** in a 2:1 ratio as judged by ^{1}H NMR analysis of the crude reaction mixture (Scheme 1). After careful silica gel chromatography, **3** and **4** were isolated in 39% and 23% yield, respectively (Scheme 1). ^{1}H NMR analysis in $CDCl_3$ showed the 19,20-dihydroxylated derivative **3** to consist of a 1:1 mixture of conformers, whereas the 21,22-dihydroxylated compound **4** exists as a ca. 4:1 ratio of conformers.[19] The fact that the mixture observed for **3** by ^{1}H NMR corresponds to conformers, and not to diastereomers, was established by a combination of DQF COSY[20] and ROESY[21,22] NMR experiments. In the 2D ROESY experiment performed with **3** an exchange cross-peak was observed between the two signals corresponding to the C2 proton (δ 4.48 and 5.22 ppm), indicating the presence of two slowly equilibrating forms of the same compound. The fact that both **3** and **4** are single diastereomers was subsequently confirmed by other means (vide infra). The regiochemical outcome of this reaction was to some degree unexpected. In previous reports concerning the AD of less complex conjugated trienes, the internal double bond remained unaffected, possibly because its dihydroxylation would result in complete disruption of conjugation.[12c] In the present case, the product resulting from dihydroxylation of the internal olefin predominated. The trisubstituted C17,C18 double bond remained untouched, although this type of olefin is generally an excellent substrate for the AD. This is probably due in large part to the electron-withdrawing inductive effect of the C16-methoxy group and also, to some degree, to its steric demands. Electronegative allylic atoms, i.e., oxygen and nitrogen, have been reported to decrease the hydroxylation rate in some cases.[23] This observation is confirmed by AD reactions performed on the diene geraniol and some of its derivatives. In those cases, dihydroxylation occurs with high preference on the trisubstituted olefin which is remote from the allylic O-substituent, again indicating a deactivating effect of the latter.[12b,13,14a] As far as the slight selectivity for the formation of **3** over **4** is concerned, it can be explained by the fact that the C21,C22 double bond is to some degree shielded by the C23-methyl group. The isolated trisubstituted C29,C30 olefin remained unchanged. This could be due in part to a deactivating effect of the allylic hydroxyl, as discussed above. But in that particular case, it is more likely that the inaccessibility of this olefin due to steric factors is the main reason for the lack of reactivity[24] (Figure 1). These structural features of the macrolide can explain the unusual regiochemical outcome of the AD.

When **2** was submitted to the reaction conditions described in eq 1, but using the pseudoenantiomeric $(DHQ)_2$PHAL instead of $(DHQD)_2$PHAL, practically no conversion was observed. Forcing the reaction conditions by increasing the temperature to 23 °C and by prolonging the reaction time also was to no avail, but rather led to some decomposition of starting material. On the basis of the Sharpless mnemonic device, $(DHQ)_2$PHAL was expected to induce dihydroxylation of the "top" face of the triene subunit of either **1** or **2** (vide supra), which is oriented toward the inside of the macrocycle and is therefore not readily accessible (Figure 1). This was predicted to be a very disfavored process. The virtual absence of reaction in the presence of $(DHQ)_2$PHAL clearly showed that dihydroxylation of the "top" face of the triene is not feasible. This result confirmed the stereochemical outcome of the reaction depicted in eq 1.

Deprotection of **3**, using HF–pyridine in acetonitrile, proceeded uneventfully to afford 19(*R*),20(*R*)-dihydroxyrapamycin **5** in 61% yield (Scheme 2). When **4** was treated in the same conditions, desilylation occurred with concomitant condensation of the 21,22-diol with the C26-ketone to provide ketal **6** as the sole product (Scheme 3).

This latter compound provided, in addition to the reactivity pattern discussed above, another opportunity for verifying the stereochemical outcome of the dihydroxylation. Inspection of Dreiding models revealed that intramolecular ketalization of the 21(*R*),22(*R*)-diol with the C26-ketone can only result in the ketal **6** having the *S* configuration at C-26. The 21(*S*),22(*S*)-diol, on the other

(15) Kwong, H.-L.; Sorato, C.; Ogino, Y.; Chen, H.; Sharpless, K. B. *Tetrahedron Lett.* **1990**, *31*, 2999–3002.

(16) Kolb, H. C.; Andersson, P. G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1994**, *116*, 1278–1291.

(17) The use of bissilylated rapamycin **2** was made necessary by the fact that unprotected rapamycin is insoluble in the biphasic *tert*-butyl alcohol:water mixture recommended for these reactions. Preclusion of the "second cycle" and of polyhydroxylation calls for such a biphasic solvent system. Attempts to use nonwater miscible organic solvents capable of solubilizing rapamycin, such as methylene chloride, toluene, or diethyl ether, led to no reaction.

(18) For a standard procedure, see: Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768–2771. In this study we added the individual components separately instead of using the premixed powder. Instead of potassium osmate, osmium tetraoxide solution was used. More importantly, the loads of osmium catalyst and phthalazine ligand were 5-fold higher than those recommended in the published standard procedure (see Experimental Section for more details).

(19) Rapamycin itself exists in $CDCl_3$ as a mixture of conformers in a ratio of about 3:1: McAlpine, J. B.; Swanson, S. J.; Jackson, M.; Whittern, D. N. *J. Antibiot.* **1991**, *44*, 688–690.

(20) Rance, M.; Sorensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479–485.

(21) Bothner-By, A. A. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.

(22) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207–213.

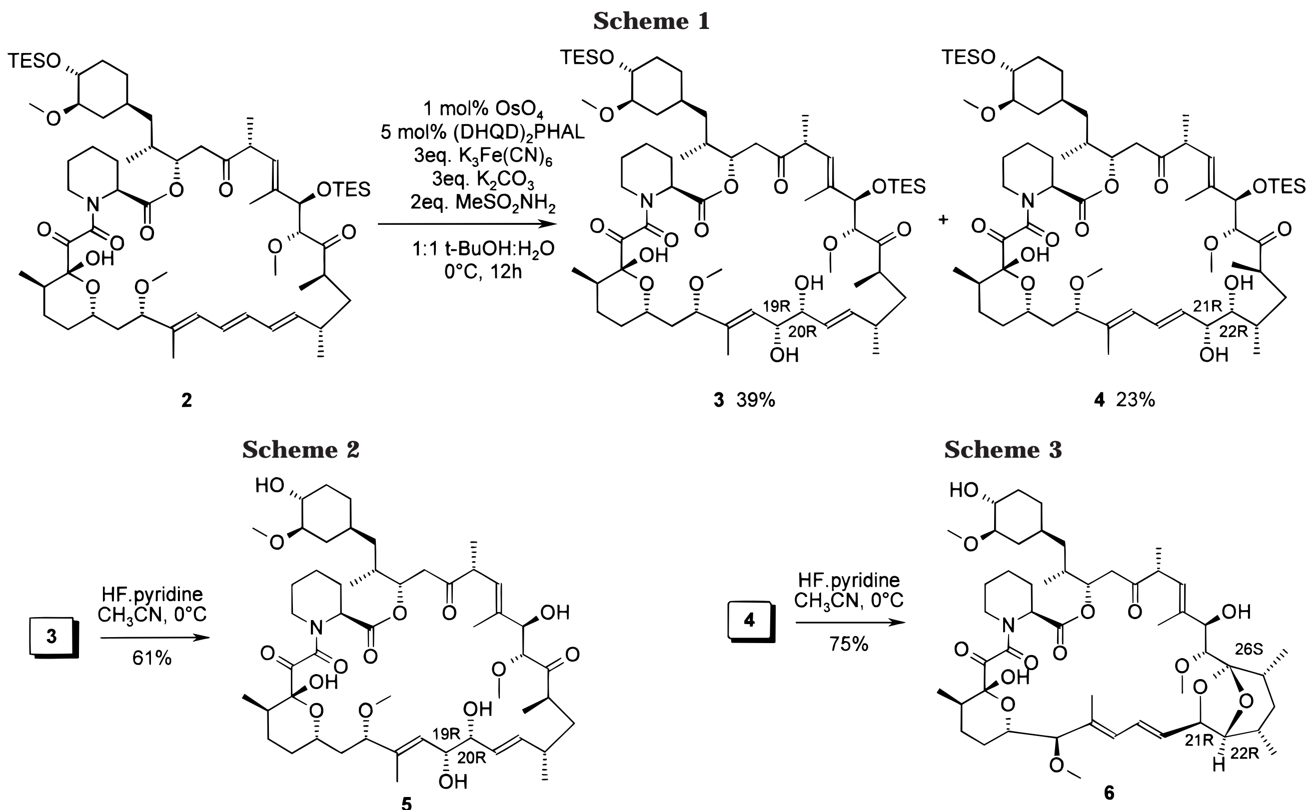
(23) Wade, P. A.; D'Ambrosio, S. G.; Rao, J. A.; Shah-Patel, S.; Cole, D.T; Murray, J. K., Jr.; Carroll, P. J. *J. Org. Chem.* **1997**, *62*, 3671–3677 and references therein.

(24) Hydrogenation of rapamycin over Pd/C results in complete saturation of the triene, but the C29,C30 double bond remains unchanged. This result supports the argument that the latter olefin experiences severe steric hindrance. Dr. T. Fehr from our laboratories, unpublished results.

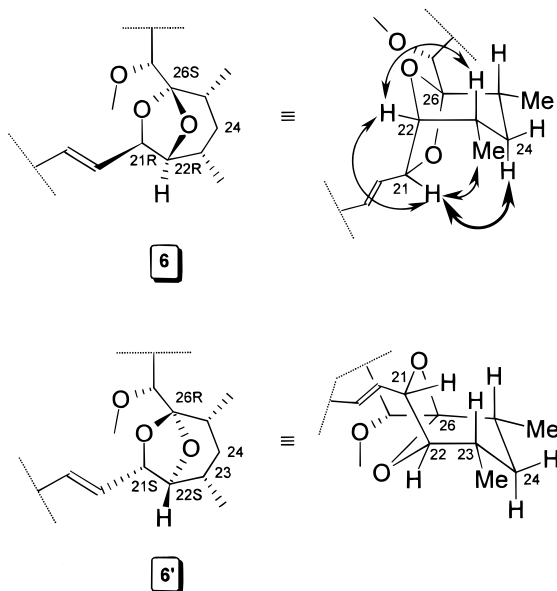
Scheme 1

Scheme 2

Scheme 3



hand, would have resulted in the 26(*R*)-ketal **6′**, of which the most likely conformation is depicted below. Strong NOEs found in the ROESY spectrum are indicated by arrows. These are in complete agreement with the 21(*R*),22(*R*),26(*S*) configurations and, thus, with structure **6**. Most notably, a NOE from H-21 to the axial H-24 is observed. This effect would not be expected for the conformation shown for **6′**. Inversely, the latter conformation should result in a NOE between H-21 and H-23, which is not observed. These NMR data confirm the structure of ketal **6** and, thereby, the stereochemical outcome of the dihydroxylation of the C21,C22 olefin.



In summary, we have shown that the AD of 28,40-*O*-bis(triethylsilyl)rapamycin **2**, using $(DHQD)_2PHAL$ as a chiral ligand, leads to a separable mixture of two regioisomeric diols **3** and **4**. The stereochemical course of the reaction, predicted on the basis of both the conformation of rapamycin and the choice of the chiral ligand, was confirmed by the virtual absence of reaction when the pseudoenantiomeric ligand $(DHQ)_2PHAL$ was used, as well as by NMR analysis of the ketal **6**. The AD reaction thus proves to be a very useful tool for selectively functionalizing the triene region of the complex natural product rapamycin. Moreover, the chemistry described herein allows to prepare a rapamycin metabolite, or at least one of its stereoisomers, in sufficient amounts to evaluate the effect of triene-dihydroxylation on the immunosuppressive activity. The biological activities of the compounds described in this paper and of derivatives are going to be described elsewhere.

Experimental Section

All reagents and solvents were used as received from the suppliers without further purification. Rapamycin was obtained from Novartis Pharma Research, Core Technology Area, Biomolecules Production, Basel, Switzerland. Osmium tetraoxide (2.5 wt % solution in *tert*-butyl alcohol), $(DHQ)_2PHAL$, and $(DHQD)_2PHAL$, were purchased from Aldrich. Analytical thin-layer chromatography was performed with precoated glass-backed plates (Merck Kieselgel 60 F_{254}). Compounds were purified by flash chromatography[25] using Merck Kieselgel 60 (40–63 μm). ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz, respectively.

28,40-*O*-Bis(triethylsilyl)rapamycin (2). To a stirred, cooled (0 °C) solution of rapamycin (50.0 g, 54.68 mmol) and imidazole (18.61 g, 273.43 mmol) in 280 mL of DMF was added triethylchlorosilane (20.2 mL, 120.30 mmol) over 5 min. The resulting yellow solution was stirred at 0 °C for 1.5 h, and the reaction mixture was quenched by slow addition of 300 mL of saturated aqueous sodium bicarbonate, which was accompanied by sig-

(25) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

nificant gas evolution. Then 400 mL of ethyl acetate were added, and the layers were separated. The organic layer was washed successively with 200 mL of saturated aqueous sodium bicarbonate, twice with 200 mL of water, and finally twice with 200 mL of saturated brine. The aqueous solutions were combined and extracted twice with 500 mL of ethyl acetate. The combined organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (60:40 hexanes/methyl *tert*-butyl ether) to afford bis-O-silylated rapamycin **2** (56.6 g, 90%) as an off-white amorphous solid: ^{1}H NMR ($CDCl_3$) (~4:1 mixture of conformers, only signals of major conformer listed) δ 0.54 (m, 6H), 0.64 (m, 6H), 0.73 (dd, J = 12.1, 23.9 Hz, 1H), 0.84−1.03 (m, 24H), 1.04−1.13 (m, 9H), 1.13−1.30 (m, 4H), 1.32−1.47 (m, 4H), 1.48−1.72 (m, 8H), 1.68 (s, 3H), 1.78 (s, 3H), 1.73−1.97 (m, 5H), 2.02 (m, 2H), 2.30 (m, 2H), 2.37 (dd, J = 2.5, 15.6 Hz, 1H), 2.63 (dd, J = 8.0, 15.6 Hz, 1H), 2.73 (m, 1H), 2.93 (m, 1H), 3.16 (s, 3H), 3.29 (s, 3H), 3.32−3.43 (m, 3H), 3.45 (s, 3H), 3.72 (m, 2H), 3.81 (m, 1H), 3.88 (d, J = 6.3 Hz, 1H), 4.16 (d, J = 5.9 Hz, 1H), 4.73 (s, 1H), 5.06 (m, 1H), 5.26 (d, J = 10.2 Hz, 1H), 5.34 (d, J = 4.7 Hz, 1H), 5.59 (dd, J = 8.0, 15.0 Hz, 1H), 6.08 (d, J = 10.8 Hz, 1H), 6.19 (dd, J = 10.3, 15.0 Hz, 1H), 6.34 (dd, J = 10.3, 14.6 Hz, 1H), 6.44 (dd, J = 10.8, 14.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) (~4:1 mixture of conformers, only signals of major conformer listed) δ 4.6, 4.7, 5.0, 6.6, 6.7, 6.8, 10.1, 12.3, 13.7, 14.9, 15.4, 16.0, 20.4, 21.4, 25.1, 26.9, 27.3, 31.3, 31.8, 33.0, 33.8, 33.9, 34.1, 35.2, 36.1, 38.5, 38.6, 39.8, 41.7, 42.4, 44.0, 47.0, 51.2, 55.8, 58.0, 58.1, 66.9, 75.6, 76.8, 77.2, 79.2, 84.0, 84.1, 84.7, 98.6, 126.8, 127.1, 129.4, 130.7, 132.8, 135.8, 138.1, 139.1, 166.3, 169.6, 193.4, 208.4, 211.5; IR (KBr) 1109, 1648, 1724 cm^{-1}; MS (FAB, nitrobenzyl alcohol + lithium iodide matrix) calcd for $C_{63}H_{107}NO_{13}Si_2Li$ 1148, found 1148; Anal. Calcd for $C_{63}H_{107}NO_{13}Si_2$ C, 66.22%; H, 9.44%; N, 1.23%. Found C, 65.80%; H, 9.29%; N, 1.12%; R_f 0.32 (60:40 hexanes/methyl *tert*-butylether); $[\alpha]^{25}_D$ = −112.1 (c 0.26, $CHCl_3$).

28,40-*O*-Bis(triethylsilyl)-19(*R*),20(*R*)-dihydroxyrapamycin (3) and 28,40-*O*-Bis(triethylsilyl)-21*(R)*,22(*R*)-dihydroxyrapamycin (4). In a mixture of 10 mL *tert*-butyl alcohol and 20 mL of water was dissolved, under stirring, potassium ferricyanide (1.98 g, 6.00 mmol), potassium carbonate (0.84 g, 6.00 mmol), methanesulfonamide (0.38 g, 4.00 mmol), $(DHQD)_2PHAL$ (78 mg, 0.10 mmol), and osmium tetraoxide (0.26 mL of a 2.5 wt % solution in *tert*-butyl alcohol, 0.02 mmol). The resulting yellow emulsion was cooled to 0 °C, and a solution of **2** (2.28 g, 2.00 mmol) in 10 mL of *tert*-butyl alcohol was added. Stirring was continued at 0 °C for 12 h. After this time TLC analysis indicated complete consumption of the starting material and the appearance of two new polar products. To the reaction mixture was added 40 mL of 1 N aqueous sodium bicarbonate solution, and the resulting emulsion was poured into an Erlenmeyer flask containing 100 mL of methyl *tert*-butyl ether. After stirring for 15 min, the organic layer was transferred to another Erlenmeyer flask, charged with solid sodium sulfite (3.00 g, 23.00 mmol). Water was added until the salt had dissolved, and the mixture was stirred for 0.5 h. The layers were separated, and the aqueous solution was extracted once with methyl *tert*-butyl ether. The combined organic solution was washed with saturated aqueous sodium bicarbonate and saturated brine, then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was carefully purified by column chromatography on silica gel (80:20−70:30 hexanes/acetone) to afford the 19,20-dihydroxy derivative **3** (910 mg, 39%) and the 21,22-dihydroxy derivative **4** (544 mg, 23%) as white amorphous solids.

3: ^{1}H NMR ($CDCl_3$) (~1:1 mixture of rotamers) δ 0.46−0.65 (m, 12H), 0.67 (m, 1H), 0.82−1.00 (m, 24H), 1.01−1.21 (m, 12H), 1.22−1.50 (m, 6H), 1.51−1.71 (m, 9H), 1.72−1.86 (m, 7H), 1.91−2.06 (m, 3H), 2.11−2.29 (m, 3H), 2.50 (dd, J = 4.8, 17.1 Hz, 0.5 H), 2.66−2.93 (m, 3.5H), 3.10−3.22 (m, 5H), 3.31−3.56 (m, 9.5H), 3.62 (dd, J = 5.3, 10.5 Hz, 0.5H), 3.72−3.82 (m, 2.5H), 4.04 (bm, 1H), 4.26 (2d, J = 5.8 and 7.2 Hz, 1H), 4.40 (m, 1H), 4.45 (broad, 0.5H), 4.48 (d, J = 3.8 Hz, 0.5H), 5.15 (m, 1H), 5.22 (d, J = 4.8 Hz, 0.5H), 5.33 (d, J = 9.6 Hz, 0.5H), 5.40 (d, J = 7.9 Hz, 0.5H), 5.43 (d, J = 9.9 Hz, 0.5H), 5.52 (d, J = 4.8 Hz, 1H), 5.56 (d, J = 4.8 Hz, 0.5H), 5.63 (d, J = 8.2 Hz, 0.5H), 5.79 (m, 1.5H); ^{13}C NMR ($CDCl_3$) (~1:1 mixture of rotamers, some signals overlap) δ 4.6, 4.7, 5.0, 6.7, 6.8, 10.1, 10.5, 11.9, 12.2; 15.2, 15.6, 15.8, 16.0, 16.2, 16.3, 16.4; 20.7, 21.4; 21.5, 24.3, 25.0, 26.8, 26.9, 27.3, 28.3, 31.7, 31.9, 32.0, 32.9, 33.0, 33.2, 33.4, 33.7, 34.0, 34.4, 34.9, 35.0, 36.0, 38.1, 38.3, 38.7, 39.6, 40.9, 41.0, 42.4, 42.7, 44.1, 46.9, 47.2, 51.5, 55.8, 56.1, 58.1, 58.2, 59.0, 66.5, 70.2, 70.4, 74.7, 75.1, 75.6, 75.7, 75.8, 77.5, 78.2, 83.5, 84.1, 84.3, 85.5, 98.1, 99.1, 126.9, 127.6, 127.9, 128.3, 130.0, 131.5, 133.5, 135.3, 137.1, 137.2, 137.9, 138.2, 166.0, 166.6, 169.6, 169.8, 194.5, 197.1, 208.6, 208.8, 210.9, 211.3; IR (KBr) 1108, 1651, 1722, 3439 cm^{-1}; MS (FAB, nitrobenzyl alcohol + lithium iodide matrix) calcd for $C_{63}H_{109}NO_{15}Si_2Li$ 1182, found 1182. Anal. Calcd for $C_{63}H_{109}NO_{15}Si_2$ C, 64.30%; H, 9.34%; N, 1.19%. Found C, 63.91%; H, 9.20%; N, 1.12%; R_f 0.35 (70:30 hexanes/acetone); $[\alpha]^{25}_D$ = −54.6 (c 0.25, $CHCl_3$).

4: ^{1}H NMR ($CDCl_3$) (~4:1 mixture of rotamers, only signals corresponding to major rotamer listed) δ 0.49−0.72 (m, 13H), 0.84−1.11 (m, 30H), 1.12−2.08 (m, 33H), 2.32 (bd, J = 13.7 Hz, 1H), 2.65 (dd, J = 4.5, 13.7 Hz, 1H), 2.76 (m, 2H), 3.00 (bs, 1H), 3.11 (s, 3H), 3.24−3.36 (m, 5H), 3.37−3.62 (m, 6H), 3.63−3.80 (m, 3H), 4.12 (t, J = 8.23 Hz, 1H), 4.30 (d, J = 7.9 Hz, 1H), 5.05 (s, 1H), 5.23 (bd, J = 5.1 Hz, 1H), 5.30 (m, 1H), 5.42 (d, J = 8.6 Hz, 1H), 5.63 (dd, J = 7.7, 15.0 Hz, 1H), 5.96 (d, J = 11.0 Hz, 1H), 6.59 (dd, J = 11.0, 15.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) (~4:1 mixture of rotamers, only signals corresponding to major rotamer listed) δ 4.6, 5.0, 6.7, 6.8, 9.9, 11.7, 13.9, 16.4, 16.7, 17.1, 17.5, 20.6, 25.4, 26.9, 27.3, 27.6, 31.4, 32.2, 32.8, 33.2, 34.0, 34.1, 35.7, 36.6, 37.3, 38.1, 40.7, 44.1, 45.2, 46.8, 51.0, 55.8, 57.9, 58.1, 60.0, 66.7, 73.3, 74.7, 75.5, 75.7, 83.9, 84.2, 84.5, 98.1, 127.8, 129.4, 131.8, 135.9, 138.4, 167.1, 168.9, 191.5, 208.7, 212.8; IR (KBr) 1106, 1652, 1719 3442 cm^{-1}; MS (FAB, nitrobenzyl alcohol + lithium iodide matrix) calcd for $C_{63}H_{109}NO_{15}Si_2Li$ 1182, found 1182. Anal. Calcd for $C_{63}H_{109}NO_{15}Si_2$ C, 64.30%; H, 9.34%; N, 1.19%. Found C, 64.13%; H, 9.20%; N, 1.12%; R_f 0.30 (70:30 hexanes/acetone); $[\alpha]^{25}_D$ = −33.9 (c 0.25, $CHCl_3$).

19(*R*),20(*R*)-Dihydroxyrapamycin (5). To a stirred, cooled (0 °C) solution of **3** (353 mg, 0.30 mmol) in 3 mL of acetonitrile was added 0.3 mL of HF−pyridine complex. Stirring was continued for 2 h at 0 °C, and the reaction was quenched by the addition of 1 N aqueous sodium bicarbonate. The resulting mixture was extracted twice with methyl *tert*-butyl ether. The combined organic solution was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (50:50−40:60 hexanes/acetone) to give **5** (180 mg, 63%) as a white amorphous solid: ^{1}H NMR ($CDCl_3$) (~2:1 mixture of rotamers) δ 0.66 (m, 1H), 0.80 (d, J = 6.6 Hz, 3H), 0.84−1.87 (m, 37H), 1.96 (bm, 2H), 2.13 (bm, 2H), 2.26 (bm, 2H), 2.50−2.80 (m, 2H), 2.81−3.04 (m, 2H), 3.07−3.23 (m, 6H), 3.30−3.86 (m, 12H), 4.06 (bm, 1H), 4.25 (d, J = 6.2 Hz, 1H), 4.40 (m, 3H), 5.00 (bm, 1H), 5.16 (bm, 1H), 5.40−5.51 (m, 1.5H), 5.54−5.70 (m, 1.5H), 5.75 (m, 1H), 6.08 (dd, J = 6.6, 15.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) (~2:1 mixture of rotamers) δ 10.0, 10.8, 13.6, 14.1, 15.1, 15.6, 15.8, 16.1, 16.2, 16.6, 17.0, 17.8, 20.6, 20.8, 21.0, 21.4, 24.1, 26.6, 26.7, 26.9, 27.3, 28.0, 29.2, 31.3, 31.6, 31.7, 31.8, 32.4, 33.1, 33.2, 33.4, 33.7, 33.9, 34.1, 35.0, 38.1, 38.2, 38.8, 38.9, 39.0, 39.7, 40.0, 40.5, 40.6, 40.8, 44.0, 45.9, 46.4, 49.4, 51.5, 53.7, 55.9, 56.0, 56.6, 58.8, 66.4, 66.7, 70.2, 73.9, 74.5, 75.4, 75.7, 83.5, 84.4, 86.1, 87.9, 98.1, 99.3, 125.2, 125.8, 127.0, 128.6, 129.8, 131.9, 133.1, 135.2, 136.2, 136.5, 138.9, 166.1, 166.9, 169.3, 170.1, 194.6, 197.7, 209.2, 210.4, 213.7, 215.1; IR (KBr) 1093, 1648, 1718, 3447 cm^{-1}; MS (ESI) calcd for $C_{51}H_{81}NNaO_{15}$ 970, found 970. Anal. Calcd for $C_{51}H_{81}NO_{15}$ C, 64.60%; H, 8.61%; N, 1.48%. Found C, 64.36%; H, 8.46%; N, 1.21%; R_f 0.37 (40:60 hexanes/acetone); $[\alpha]^{25}_D$ = −28.5 (c 0.26, $CHCl_3$).

Ketal (6). To a stirred, cooled (0 °C) solution of **4** (206 mg, 0.17 mmol) in 2 mL of acetonitrile was added 0.2 mL of HF−pyridine complex. Stirring was continued for 1.5 h at 0 °C, and the reaction was quenched by the addition of 1 N aqueous sodium bicarbonate. The resulting mixture was extracted twice with methyl *tert*-butyl ether. The combined organic solution was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (70:30−60:40 hexanes/acetone) to give **6** (118 mg, 75%) as a white amorphous solid:^{1}H NMR ($CDCl_3$) δ 0.75 (dd, J = 11.8, 23.8 Hz, 1H), 0.93 (m, 9H), 1.01 (d, J = 6.4 Hz, 3H), 1.03−1.17 (m, 2H), 1.24 (d, J = 6.8 Hz, 3H), 1.27−1.81 (m, 24H), 1.88−2.22 (m, 6H), 2.33 (bd, J = 13.7 Hz, 1H), 2.44 (dd, J = 2.3, 16.4 Hz, 1H), 2.66 (s, 1H), 2.85 (dd, J = 8.1, 16.4 Hz, 1H), 2.99 (m, 1H), 3.17 (s, 3H), 3.26

(d, $J = 8.0$ Hz, 1H), 3.38−3.48 (m, 8H), 3.51−3.65 (m, 3H), 3.78 (bt, $J = 11.2$ Hz, 1H), 3.87 (dd, $J = 4.7$, 11.1 Hz, 1H), 4.10 (d, $J = 2.1$ Hz, 1H), 4.52 (d, $J = 9.6$ Hz, 1H), 4.62 (d, $J = 7.8$ Hz, 1H), 5.41 (m, 1H), 5.47 (d, $J = 4.5$ Hz, 1H), 5.57 (d, $J = 1.4$ Hz, 1H), 5.64 (d, $J = 8.8$ Hz, 1H), 6.03 (d, $J = 10.8$ Hz, 1H), 6.10 (dd, $J = 9.8$, 15.1 Hz, 1H), 6.39 (dd, $J = 10.8$, 15.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.6, 15.3, 16.5, 16.7, 17.0, 17.7, 21.0, 25.8, 27.2, 29.2, 31.3, 31.4, 31.6, 32.5, 33.1, 33.3, 33.6, 34.1, 34.4, 34.8, 38.3, 39.4, 39.7, 44.0, 46.3, 51.1, 55.8, 56.5, 60.9, 66.8, 71.2, 71.4, 73.9, 74.8, 77.8, 83.8, 83.9, 84.4, 85.2, 98.6, 111.7, 122.6, 127.5, 128.6, 132.7, 136.3, 142.0, 167.1, 168.5, 190.0, 208.0; IR (KBr) 1099, 1624, 1744, 3503; MS (FAB, nitrobenzyl alcohol + lithium iodide matrix) calcd for $C_{51}H_{79}LiO_{14}$ 936, found 936. Anal. Calcd for $C_{51}H_{79}NO_{14}$ C, 65.85%; H, 8.56%; N, 1.51%. Found C, 65.43%; H, 8.38%; N, 1.32%; R_f 0.21 (60:40 hexanes/acetone); $[\alpha]^{25}{}_D = -57.0$ (c 0.26, $CHCl_3$).

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for compounds **2**−**6** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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